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Total Hexane and Ethanol Extractives of Tobacco

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ABSTRACT

A composition study was made of the total n-hexane and ethanol extractives of unaged, cured Type 12 tobacco. The nature and amounts of the substances therein are described.

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TOTAL HEXANE AND ETHANOL EXTRACTIVES OF TOBACCO

by

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A composition study was made of the substances removed from flue-cured tobacco by large-scale serial extraction with n-hexane and ethanol. The composition of the n-hexane extractives has been described (10, 11).[1] The nature and amounts of the substances in the ethanol extract and a material balance of all extractives are given in this report.

As previously described (2), 39.6 kg. of ground (10 mesh) leaf webs of unaged, cured Type 12 tobacco (mixed grades) were serially extracted by n-hexane and ethanol. After the ethanol extract was concentrated and refrigerated at 0° C., it gave a precipitate (EP1) and filtrate (E1). An outline of the subsequent fractionation is given in figure 1. After removal of the water solubles in EP1, the water insolubles were fractionated into neutral and acidic and basic substances. The neutrals were separated, as previously outlined (9), and gave campesterol, the methyl and ethyl esters of higher fatty acids, and neutral resins. The acids and bases were not separated further.

On continued storage at 0° C., fraction E1 yielded a precipitate (E2), which was removed by centrifugation. The precipitate showed an infrared spectrum identical with that of sodium propionate. It was tentatively identified as a salt (or mixture of salts) of propionic or other lower fatty acids or both. To the decantate (E3) was added sufficient Skellysolve B[2] to give two layers (1 part E3:2 parts Skellysolve). The layers were then separated and to the lower layer were added sufficient ethanol (8.47 liters) and Skellysolve (29.5 liters) to give two layers, and the layers were again separated. A further addition of the same amounts of ethanol and Skellysolve B to the resulting lower layer again gave two layers, which were separated. This final lower layer was E4. The pooling of all upper layers gave E5.

E5 was concentrated and the precipitate (E6) that formed was filtered off. The filtrate was evaporated to a residue, which was impregnated on

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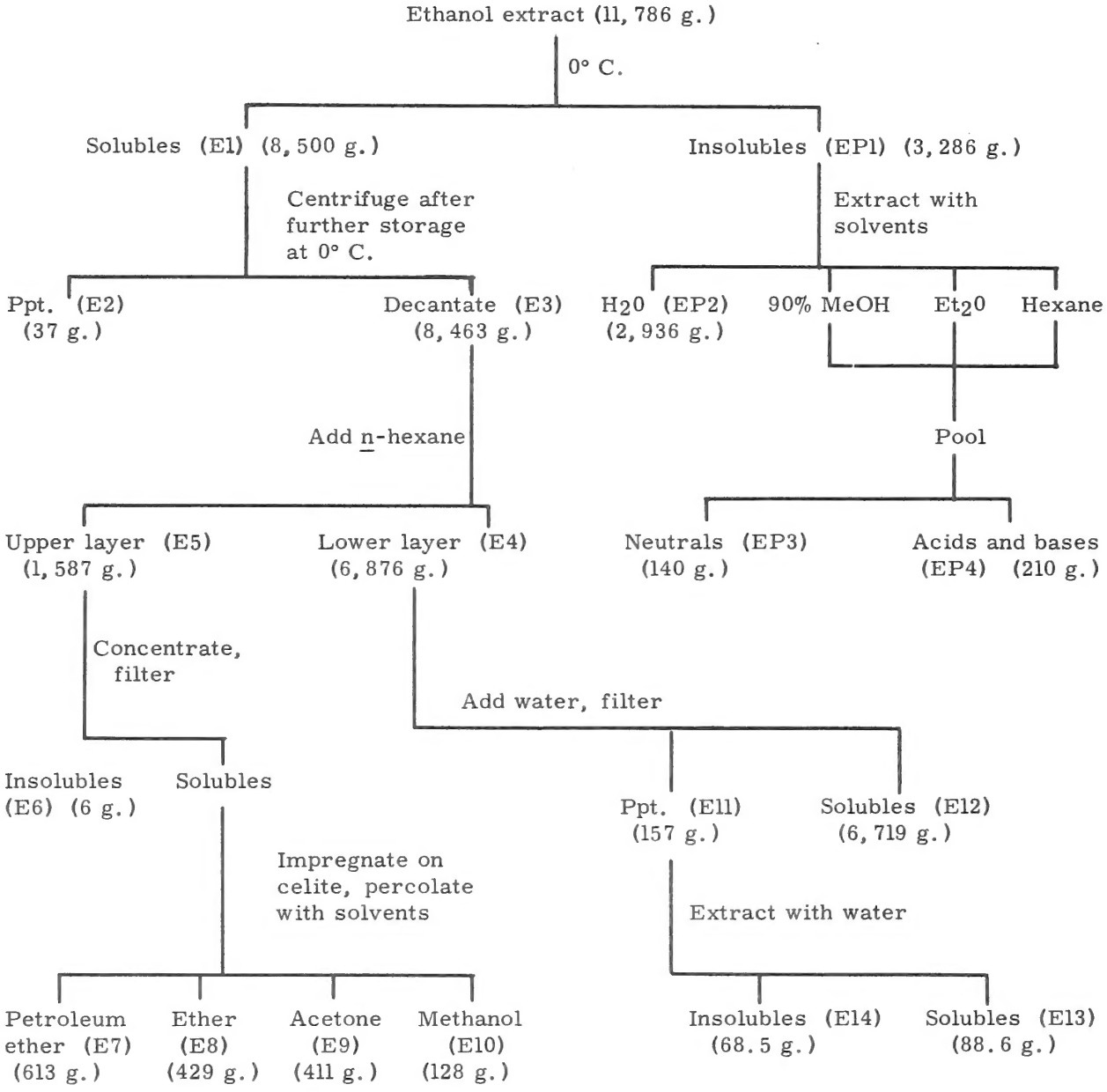
[1] Numbers in parentheses refer to literature cited at the end of this report

[2] Essentially n-hexane. Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture over similar products not named.

Figure 1.--Fractionation of ethanol extract.
(Weights not corrected for moisture in tobacco)



celite (1 part residue:2.1 parts celite, w/w). The dried residue-celite mixture was packed in a chromatographic column and successively percolated with petroleum ether, ether, acetone, and methanol. In each case, fresh solvent was continually passed through the column until the weight of material removed from the column became negligible. This entire procedure permitted gross separation of E3 on the basis of relative solubilities. The petroleum ether (E7) and ether (E8) percolates were then extensively studied after initial separation of the residues of each percolate into neutral and acidic and basic substances. The methods of isolation and identification were similar to those described previously (2, 9, 10). The residues from the acetone (E9) and methanol (E10) percolates were found to contain mostly water-soluble materials, which were not of interest in this study, and extensive attempts to identify the relatively small amounts of water-insoluble substances were not made.

The lower layer (E4) was diluted fourfold with water. After refrigeration at 5° C. for 1 month, the mixture was filtered through celite and gave a filtrate (E12) and a precipitate (E11) on celite. The precipitate-celite mixture was exhaustively extracted with methanol and the methanol extracts were evaporated to a residue. Extraction of the residue with water gave water-soluble (E13) and water-insoluble (E14) fractions.

Table 1 shows the weights and composition of the water-insoluble substances found in the fractions. Table 2 gives the total amounts of water-soluble and water-insoluble substances in the ethanol extractives. The weights and composition of the ethanol extractives are given in table 3. More than 92 percent of the ethanol extractives consisted of water-soluble material.

The final compilation of the composition of total n-hexane and ethanol extractives is shown in table 4. The values are given to the third decimal place to show relative quantities isolated, and no statistical significance is intended. Actually all values should be considered as semiquantitative estimates, since the isolation methods are not analytical procedures. The nature of the paraffins, neophytadiene, polyene, phthalates, sterols, sterol glycosides, tocopherols, fatty acid salts, higher fatty acids, neutral resins, and substances not eluted from silicic acid or alumina columns with methanol have been reported (2, 9, 10, 11, 12, 13). Solanesol (3, 5) and scopoletin (6) are well-known components of tobacco. The "Fatty Acid Esters" in the hexane extract (10, 11) and, in part, the ethanol extract (9) have also been reported. The "Unidentified Acids" consisted mainly of either thick, highly colored resins or black, amorphous tars, which showed infrared spectra, with strong absorption for the carboxyl and carbonyl groups. Perhaps much of this material could be described as acidic resin (4).

Comparison of these data with the results of recent quantitative studies on tobacco constituents is of interest. Analytical methods for the determination of paraffinic hydrocarbons (8), sterols (7), higher fatty

Table 1--Weights and composition of water-insoluble substances in ethanol extractives

Substance	Weights (grams) in indicated fractions [1]									
	E2	E6	E7	E8	E9	E10	E14	EP3	EP4	Total
Aliphatic paraffins			1.6					12.9		14.5
Neophytadiene			2.0							2.0
Polyene			12							12
Fatty acid esters			88.2					18.2		106.4
Unidentified esters				8						8
Sterols								1.5		1.5
Sterol glycosides		6		5	0.2					11.2
Fatty acid salts	37									37
Unidentified salts				15.5						15.5
Scopoletin				1						1
Unidentified acids			23.8	55	12.1					90.9
Other unidentified acids and bases									210	210
Bases			0.5	2						2.5
Other unidentified substances						5.8	68.5			74.3
Resins (neutral)			155	8	7.6			107		277.6
Unidentified substances not eluted with methanol			40							40
Total	37	6	323.1	94.5	19.9	5.8	68.5	139.6	210	904.4

[1] Not corrected for moisture in tobacco.

Table 2--Weights of water-soluble and water-insoluble substances in ethanol extractives [1/]

Fraction	Weight (grams)		
	Water solubles	Water insolubles	Total [2/]
E2	0	37	37
E6	0	6	6
E7	290	323.1	613.1
E8	335	94.5	429.5
E9	390	19.9	409.9
E10	123	5.8	128.8
E12	6,719	0	6,719
E13	88.6	0	88.6
E14	0	68.5	68.5
EP2	2,936	0	2,936
EP3	0	139.6	139.6
EP4	0	210	210
Total	10,881.6	904.4	11,786

[1/] Not corrected for moisture in tobacco.

[2/] Small differences (usually <1.0 g.) between totals here and weights of original fractions (fig. 1) are attributed to such factors as losses during extensive manipulation or failure to eliminate all traces of solvent before weighing.

Table 3--Content of substances in ethanol extractives and in tobacco

Substance	Content (%)		
	In ethanol extractives	In tobacco	In tobacco [1]
Aliphatic paraffins	0.123	0.0366	0.040
Neophytadiene	.017	.0051	.006
Polyene	.102	.0303	.033
Fatty acid esters	.903	.2687	.296
Unidentified esters	.068	.0202	.022
Sterols	.013	.0038	.004
Sterol glycosides	.095	.0283	.031
Fatty acid salts	.314	.0934	.103
Unidentified salts	.132	.0391	.043
Scopoletin	.009	.0025	.003
Unidentified acids	.771	.229	.252
Other unidentified acids and bases	1.78	.5303	.583
Bases	.021	.0063	.007
Other unidentified substances	.630	.1877	.206
Resins (neutral)	2.36	.701	.771
Unidentified substances not eluted with methanol	.339	.101	.111
Water solubles	92.33	27.48	30.23
Total	100.0	29.76	32.74

[1] Corrected for moisture. All other values are uncorrected for moisture.

Table 4--Composition of total n-hexane and ethanol extractives in Type 12 tobacco [1/]

Substance	Percent in Tobacco		
	n-Hexane extractives	Ethanol extractives	Total
Aliphatic paraffins	0.197	0.040	0.237
Neophytadiene	.028	.006	.034
Cyclic paraffins	.108	0	.108
Polyene	.110	.033	.143
Fatty acid esters	.203	.296	.499
Unidentified esters	0	.022	.022
Phthalates	.018	0	.018
Solanesol	.030	0	.030
Sterols	.037	.004	.041
Sterol glycosides	0	.031	.031
Tocopherols	.069	0	.069
Fatty acid salts	.015	.103	.118
Unidentified salts	0	.043	.043
Scopoletin	0	.003	.003
Higher fatty acids	.367	0	.367
Unidentified acids	.146	.252	.398
Other unidentified acids and bases	0	.583	.583
Bases	.089	.007	.096
Other unidentified substances	.070 [2/]	.206	.276
Resins (neutral)	2.35	.771	3.12
Unidentified substances not eluted with methanol	.993	.111	1.10
Water solubles	1.48	30.23	31.71
Total	6.31	32.74	39.05

[1/] Moisture-free basis.
[2/] Substances insoluble in the concentrated extract at 0° C., including some sterols. [See Bilinsky and Stedman (1).]

acids (12), and solanesol-like substances (1) in tobacco have been developed, and the levels in various tobacco types and grades have been reported.

The values for total aliphatic paraffins in this previous analytical (0.23 percent) and the present compositional (0.24 percent) studies are similar. However, a valid comparison may be questionable here, since the response of the cycloparaffins, which were found subsequent to the development of the analytical method, is unknown. Based on available evidence, their response, if any, would appear negligible, and the analytical method measures aliphatic paraffins.

A comparison of the sterol levels is more difficult, since only an approximation of the total steryl esters of fatty acids in the n-hexane extractives can be made (11). Based on such approximation, the total (free, esterified and glycosidated) sterol level in the compositional studies is less than 0.2 percent compared to the analytical value of 0.43 percent (7). Several possible reasons for this difference are apparent, including failure to isolate all sterols in the extracts, such as those present in a small fraction of the n-hexane extract (see footnote 2, table 4), or incomplete extraction, or both. Although the overall large-scale extraction was rather thorough (2), sterols are difficult to remove from tobacco and the extraction may have been incomplete for these components.

A similar situation exists with solanesol. Although the analytical procedure (1) determines solanesol-like substances (SLS), the bulk of the material responding to the method is probably solanesol. The analytical value for this tobacco was 1.32 percent, which was about 30 times higher than the level in the compositional work, assuming 70 percent of SLS is solanesol (1). Some of this difference may be due to incomplete extraction or to the difficulties of isolating solanesol. Because solanesol is eluted very slowly from silicic acid columns, it is distributed through many fractions, with probable overall loss through dilution with other components.

The values for free higher fatty acids in the analytical study (12) (0.39 percent, moisture-free basis) and in the composition study are very similar.

Although many water-insoluble components of the hexane and ethanol extractives have been isolated and identified, most of the substances remain unidentified. Much additional work is required to fractionate the components in the ethanol extract and to determine their role, if any, in the many practical problems of tobacco utilization.

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